SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 25, No. 2, pp. 428–437, 2006
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Printed in the USA
0730-7268/06 $12.00 + .00

TREE SWALLOW (*TACHYCINETA BICOLOR*) EXPOSURE TO POLYCHLORINATED BIPHENYLS AT THE KALAMAZOO RIVER SUPERFUND SITE, MICHIGAN, USA

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(*Received* 15 *September* 2004; *Accepted* 13 *July* 2005)

Abstract—In 1990, a portion of the Kalamazoo River in Michigan, USA, was designated a Superfund site because of the presence of polychlorinated biphenyls (PCBs) in the sediment and floodplain soils. During a four-year period from 2000 to 2003, several avian species were monitored for reproductive effects and concentrations of PCBs in tissues attributed to food chain transfer from contaminated sediments. The tree swallow (*Tachycineta bicolor*) was chosen as a model receptor for contamination of passerine species. A top-down methodology was used to evaluate the bioaccumulation of PCBs, including non-*ortho* and mono-*ortho* congeners, in tree swallow eggs, nestlings, and adults at the Kalamazoo River area of concern (KRAOC) and at an upstream reference site. Generally, a sixfold difference in tissue concentrations of total PCBs was observed between the two sites with concentrations in eggs and nestlings at the KRAOC ranging from 0.95 to 15 μg PCB/g wet weight. Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQs_{WHO-Avian}$) for PCBs, based on bird-specific World Health Organization toxic equivalence factors, were 10- to 30-fold greater in the KRAOC than at the reference location. Egg and nestling $TEQs_{WHO-Avian}$ ranged from 0.21 to 2.4 ng TEQ/g wet weight at the KRAOC. Hazard quotients calculated from literature-derived toxicity reference values were below 1.0 at both the target and the reference site based on the no-observed-adverse-effect level and the lowest-observed-adverse-effect level.

Keywords—Birds Bioaccumulation Aquatic food chain Toxic equivalents Eggs

INTRODUCTION

Polychlorinated biphenyls (PCBs) were used in the production of carbonless copy paper and paper inks over approximately 15 years [1]. During this period, effluent containing PCBs was released into the Kalamazoo River during the recycling of carbonless copy paper. As a result, a 123-km portion of the Kalamazoo River in southwest Michigan was designated a Superfund site because of the presence of PCBs in fish, sediments, and floodplain soils. Polychlorinated biphenyls have been linked to adverse effects in numerous mammalian [2–4; http://www.epa.gov/region1/ge/thesite/restofriver-reports.html] and avian species [5], especially reproductive effects. Recent studies have investigated mink exposure [6] and tree swallow reproduction [7] at the Kalamazoo River Area of Concern (KRAOC), but little information was available regarding concentrations of PCBs in the tissues of passerine species. Two basic types of methodologies were employed to assess trophic transfer and ecological risk to resident avian species. The first methodology, the bottom-up, or food web, approach, predicted dietary exposure, which was compared to threshold concentrations determined in laboratory feeding studies [8]. The alternative approach, on which we report here, measures concentrations of PCBs in the tissue of the receptor and compares these to tissue-based toxicity reference values (TRVs).

Exposure to PCBs for all tissues was based on congener-specific analysis of 100 congeners and was reported as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) and total PCBs in order to assess risk to passerine populations with primarily aquatic diets on the KRAOC and to evaluate the consistency of the two methodologies for quantifying PCB exposure. Both methods are assumed to evaluate the toxicity of PCB mixtures, but each measure has its advantages and disadvantages. The TEQ method, adopted by the United States Environmental Protection Agency (U.S. EPA) [9], describes the toxic action of non-*ortho* (coplanar) and mono-*ortho* congeners, which are mediated through the aryl-hydrocarbon receptor (AhR) and additive in toxicity. Effects mediated through this pathway are expected to be critical and therefore overshadow the effects elicited through other pathways [10]. Regulations based on this pathway are therefore considered protective of all toxic responses. However, TEQs are species and endpoint specific, so uncertainties exist when extrapolating to wild species and other endpoints. A major weakness of the concept is the disregard for pathways that do not act through the AhR pathway but may contribute to the overall toxic response of the organism to the mixture [10]. The total PCB method of evaluating toxicity assumes that the toxicities of all congeners in the mixture are similar and additive, so all potential toxic contributions from individual congeners are included in the assessment. The total PCB method does not assess the toxicity of the different pathways and interactions between the pathways, so the toxic response to the total PCB concentration can only be generalized and not specifically described on the basis of the mode of action.

Both the total PCB and the TEQ method of describing concentrations of PCBs in environmental matrices were em-

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ployed to quantify contamination in an avian species, the tree swallow. The tree swallow (*Tachycineta bicolor*) was selected as a sentinel species to determine site-specific bioavailability and trophic transfer from sediments contaminated with PCBs to upper-trophic-level avian receptors. This study focused on tree swallows as model receptors of PCB accumulation in avian wildlife because of the presence of a previously established nest box population at the reference location and an abundance of tree swallows residing throughout the river basin. The tree swallow diet consists largely of emergent aquatic insects [11–14] that are in contact with sediment during large portions of their life cycle. The aquatic insects constitute an indirect exposure pathway of PCB contamination from the sediment to tree swallows, and therefore, tree swallows are suitable monitors of sediment contamination. Prior to egg laying, tree swallows spend two or more weeks constructing nests [15], and thus concentrations of PCBs in eggs, passed by maternal exposure, are related to local sources of contamination. A large database of similar studies on tree swallow exposure to PCBs and productivity exists that suggests tree swallows are both tolerant of PCB contamination [16] and adequate monitors of aquatic sources of PCBs [17–19].

This study evaluated the risk of exposure to PCBs in tree swallows through the aquatic food chain at a portion of the Kalamazoo River known to be contaminated with PCBs and at an upstream reference site unaffected by point sources and having background concentrations of PCB in sediments. Previous reports based on this study elucidate no statistical difference in productivity, fledgling success, hatching success, brood size, and the number of fledglings between the reference and KRAOC locations, but clutch size was significantly greater at the reference location [7]. Here, we present an alternate top-down approach in which site-specific concentrations of PCBs (total PCBs and TEQs) in tree swallow adults, nestlings, and eggs are compared to literature-derived threshold values to estimate risk in aquatic food web–based passerine birds.

METHODS

Site details

Co-located studies of concentrations of PCBs in the tissues of birds and reproductive success were conducted at the Kalamazoo River. The Kalamazoo River is located in southwest Michigan beginning at the confluence of the north and south branches in Albion, Michigan, and flows northwest for approximately 200 km to Lake Michigan at Saugatuck, Michigan (Fig. 1). The main stem of the Kalamazoo River was dammed at 10 locations, but three of the dams were partially dismantled in 1986, exposing approximately 205 ha of former PCB-contaminated sediments that are now floodplain soils. Surveys of in-stream surface sediment (0–10 cm) indicate concentrations range from less than 0.001 μg/g PCB/g dry weight to 153 μg PCB/g dry weight with a mean concentration of approximately 3 μg PCB/g dry weight [20,21]. Surficial floodplain soils (0–25 cm) in the former impoundments range from less than 0.001 μg/g PCB/g dry weight to 85 μg PCB/g dry weight with mean values of approximately 11 μg PCB/g dry weight [22–24].

Two locations were selected to evaluate exposure to PCBs in tree swallows. In 2000, nest boxes were established within the KRAOC ($n = 68$), and additional boxes were added to an already existing nest box trail at the reference location ($n = 64$). Nest boxes were within the 100-year floodplain and not more than 200 m from the river. During the three-year study, a total of 34 tree swallow clutches were initiated at the KRAOC and 71 clutches at the reference location. The Fort Custer (FC) reference site was located upstream of known sources of PCB contamination between the villages of Augusta and Galesburg in southwest Michigan. The target study area was located entirely within the KRAOC and 67 km downstream of the reference location. The target area, the former Trowbridge Impoundment (TB), was formed in 1986 when the superstructure of the dam was removed, exposing the impoundment's former lake bottom. The former Trowbridge Impoundment stretches 7 km and encompasses 132 ha of exposed former sediments, now vegetated, and 70 ha of remaining impounded water. As one of three similar impoundment areas, the TB study area was selected as the worst-case scenario for wildlife exposure because it has the greatest surface area, total PCB mass, and mean surficial concentration of PCBs in soils (~11 μg/g dry wt) of any of the Kalamazoo River impoundment areas [23].

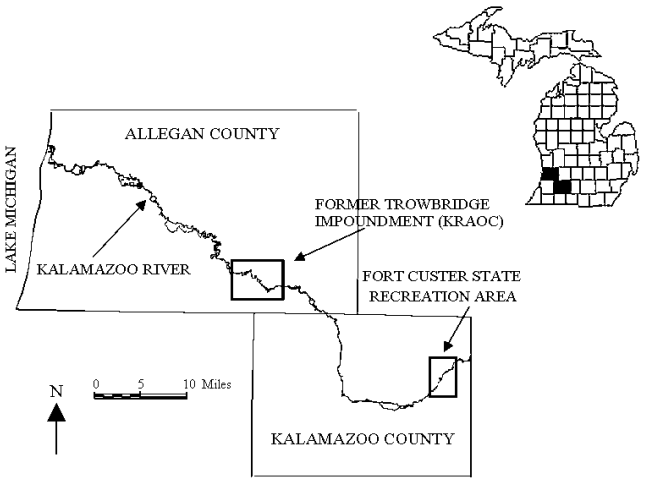

Fig. 1. Kalamazoo River area of concern (KRAOC), Michigan, USA, and the upstream reference site.

Tissue sampling

Eggs, nestlings, and adults were collected during spring and summer of 2001 and 2002. No more than one fresh egg or one live nestling was taken from any nest in a given year, and the type of sample was randomly predetermined for each box at nest initiation. Once nests were initiated, boxes were checked every 1 to 3 d to determine reproductive success. Dead adults, nestlings, and abandoned or addled eggs found in the nest boxes were salvaged and assessed for cause of death and PCB concentrations determined. Fresh eggs were preferentially sampled 10 or more days after laying. Eggs were placed in a precleaned Pelican Case® (Torrance, CA, USA) for transport back to the field laboratory, where they were placed in solvent-rinsed jars and stored at 4°C. Individual nestlings were selected randomly from predetermined broods on the day of sampling. Sampling of nestlings occurred about one week before the expected fledge date, at approximately day 12 (hatch day = 0), to allow for maximum growth while minimizing the chance that nestlings would fledge when disturbed. Nestlings were quickly removed from nest boxes, transported out of audible and visual range of the nest, and euthanized by cervical dislocation. Nestling tree swallows are sexually monochromatic, so the sex of individual nestling samples could not be determined. Chicks were placed in solvent rinsed jars and frozen at −20°C. Adults were sampled at the end of the nestling period during 2002 and 2003. Adults were captured by mist

nets erected in close proximity to the nest boxes or by a trapdoor mechanism. Adults were promptly euthanized by cervical dislocation, and sex, age, weight, body length, tarsal length, and wing chord length were determined. Sex was determined on the basis of the presence of a brood patch or cloacal protuberance. The carcasses were placed in solvent-rinsed sample jars and frozen at −20°C.

Chemical analysis

Eggs, nestlings, and adults were processed before chemical analysis. Eggshells were removed, leaving the contents of the egg, including yolk and albumen, for sample analysis. Feathers, beaks, wings, legs, and stomach contents but not skin were removed from adults and nestlings, and the whole body was homogenized in a solvent-rinsed grinder. Stomach contents were placed in cleaned vials and stored at −20°C.

Total concentrations of PCBs and of dichlorodiphenyltrichloroethane (DDT) and its metabolites were determined by U.S. EPA method 3540. All concentrations were reported as wet weight unless otherwise noted. A known quantity of tissue was homogenized with anhydrous sodium sulfate (EM Science, Gibbstown, NJ, USA) using a mortar and pestle. All samples, blanks, and matrix spikes contained surrogate standards PCB 204 (International Union of Pure and Applied Chemistry) (AccuStandard, New Haven, CT, USA) and PCB 30 (AccuStandard). Extraction blanks using Na_2SO_4 were included with each set of samples, and quality assurance/quality control sets composed of similar tissues were included with each group of 20 samples. Samples were extracted with 400 ml of pesticide residue–grade dichloromethane:hexane (3:1, volume/volume) for 18 h in a Soxhlet extraction apparatus (VWR Scientific, Plainfield, NJ, USA). Extracts were concentrated by rotary evaporation to a final volume of 11 ml. One milliliter of the hexane extract was used for lipid content determination. If deemed necessary, acid hydrolysis was performed. Briefly, 10 ml of concentrated sulfuric acid were added to the extract and shaken for at least 30 s. After separation of phases, the extract was transferred to another test tube, and 10 ml of water were added to remove acid residues from the extract. The remaining 10 ml of extract were passed through a neutral/acidic silica gel column to remove nontarget analytes. Glass columns 30 cm in length and 15 mm in diameter were packed with approximately 0.5 g anhydrous sodium sulfate and alternating layers of 2 g 40% sulfuric acid (JT Baker, Phillipsburg, NJ, USA) impregnated silica gel and 2 g 100- to 200-mesh-size silica gel (Aldrich, Milwaukee, WI, USA). The extract was then evaporated to a final volume of 1.0 ml under a stream of nitrogen. An aliquot of 0.5 ml was retained for total PCB and DDT analytes, while non-*ortho*-substituted (coplanar) PCB congeners were separated from the remaining aliquot, as described next.

Polychlorinated biphenyls including di- and mono-*ortho*-substituted congeners were quantified by use of a gas chromatograph (Perkin-Elmer AutoSystem and Hewlett-Packard 5890 series II) (Perkin-Elmer Life and Analytical Sciences, Boston, MA, USA; Agilent Technologies, Chemical Analysis Group, Wilmington, DE, USA) equipped with a ^{63}Ni electron capture detector. A fused silica capillary column (Zebron ZB-5; 5% phenylpolysiloxane, 30-m × 0.25-mm inner diameter) with a film thickness of 0.25 μm was used (Phenomenex, Torrance, CA, USA). The column oven temperature was programmed to change from 120°C (1-min hold) to 160°C at a rate of 10°C/min (1-min hold) and then to 250°C at a rate of 2°C/min with a final holding time of 10 min. Injector and detector temperatures were kept at 225 and 375°C, respectively. Helium and nitrogen were used as carrier and makeup gas, respectively. The standard contained 100 individual PCB congeners of known composition and content. Congeners were identified by comparing sample peak retention times to those of the known standard. In sample extracts, concentrations of each congener were determined by comparing the peak area to that of the appropriate peak in the standard mixture. The method detection limit was estimated to be 1×10^{-3} μg PCB/g. TurboChrom (Perkin-Elmer, Wellesley, MA, USA) or gas chromatograph Chemstation software (Agilent Technologies) was used to integrate the peaks. A spreadsheet developed by the Michigan State University Aquatic Toxicology Laboratory (East Lansing, MI, USA) was used to quantify individual congeners. Total PCB concentrations were calculated as the sum of all resolved PCB congeners.

Carbon column chromatography was used to separate non-*ortho*-substituted PCB congeners (IUPAC nos. 77, 81, 126, and 169) from coeluting congeners and interferences. Briefly, 30-cm glass columns, 15 mm in diameter, were packed with anhydrous sodium sulfate, carbon dispersed on silica gel (Wako Chemicals, Richmond, VA, USA), and anhydrous sodium sulfate. Twenty microliters of 50 μg/L isotopically ^{13}C coplanar PCB congeners (77, 81, 126, and 169) standard (Cambridge Isotope Laboratories, Andover, MA, USA) in iso-octane were added to each extract. The first fraction, eluted with 100 ml 20% dichloromethane in hexane, was archived. The second fraction, eluted with 200 ml of toluene, contained non-*ortho* coplanar PCB congeners. The extract was then concentrated under a stream of nitrogen to a final volume of 20 μl.

Concentrations of non-*ortho*-substituted PCBs and DDT isomers were quantified by gas chromatograph mass selective detector (Hewlett-Packard 5890 series II gas chromatograph) equipped with a Hewlett-Packard 5972 series detector. A fused silica capillary column (as described previously) was used. Coplanar PCB congeners and DDT isomers were detected by selected ion monitoring at the two most abundant ions of the molecular cluster. Detection limit varied among samples, but the mean detection limit for all samples was <100 pg PCB/g.

TEQ computation

Concentrations of TEQs in bird tissues were calculated by multiplying the concentration of individual PCB congeners by their respective bird-specific World Health Organization (WHO) toxic equivalence factor (TEF) [25]. Total TEQ concentrations were calculated as the sum of TEQ for non-*ortho* and mono-*ortho* PCB congeners (77, 81, 105, 118, 126, 156, 157, 167, and 169) and reported as wet weight unless otherwise noted. Polychlorinated-dibenzo-dioxins and polychlorinated-dibenzo-furans were not measured and were not included in TEQ computation. Non- and mono-*ortho* congeners are considered to have the greatest relative potency to cause effects mediated through the AhR [10,26]. A sensitivity analysis was conducted in which congeners that were not detected were assigned a proxy value equal to the detection limit or to zero [27]. The results of this analysis demonstrated that the calculated concentrations of $TEQ_{WHO-Avian}$ did not differ substantially with either proxy value. Therefore, a value of half the detection limit was assigned to congeners with concentrations below the method detection limit. Coeluting congeners were evaluated separately. For example, congener 105 coeluted with congener 132, cogener 156 frequently coeluted with congeners

171 and 202, and congener 157 frequently coeluted with congener 200. In order to report the maximum $TEQ_{WHO-Avian}$, the entire concentration of the coelution groups was assigned to the mono-*ortho* congener. Overall, congeners 105, 156, and 157 contributed little to the total concentration of the $TEQ_{WHO-Avian}$ in all samples from the Kalamazoo River, 3.7, 1.8, and 0.4%, respectively.

Biomagnification calculation

Biomagnification factors (BMFs) were calculated by dividing the lipid-normalized or wet-weight total PCB or $TEQ_{WHO-Avian}$ in the subsequent life stage by the corresponding lipid-normalized or wet-weight concentration in the previous life stage [28]. Biomagnification factors were calculated using nestlings and adults sampled live and eggs sampled fresh.

Statistical analyses

Sample sets were analyzed for normal distribution by Kolmogorov–Smirnov one-sample test with Lilliefors transformation and for homogeneity of variance by *F* test. Samples were generally log-normally distributed, and therefore all concentration data were log-transformed to obtain a normal distribution. Sample sets satisfying assumptions of normality and homogeneity were compared by *t* test or, in the case of multiple comparisons, by a one- or two-factor analysis of variance (ANOVA). All other data sets were compared by Mann–Whitney *U* or Kruskal–Wallis nonparametric tests. The criterion for significance used in all tests was $p < 0.05$.

Two eggs were sampled from three different nests. In two nests, eggs were addled, and the concentration of each egg was not more than one standard deviation different from the mean of the population. The mean concentration of PCBs in the populations were calculated on the basis of the concentrations in eggs of all other nests and the average concentration of eggs in the nests in which multiple eggs were sampled. In one nest, one egg was sampled fresh and the other addled, so these values were evaluated separately when considering differences between fresh and addled eggs, but they were averaged to calculate the mean concentration of PCBs in the population.

Assessment of risk

Risk was assessed through hazard quotients (HQs) by comparing concentrations of PCBs measured in eggs to tissue-based TRVs based on the no-observed-adverse-effect level (NOAEL) and the lowest-observed-adverse-effect level (LOAEL) for total PCBs and the NOAEL for TEQs. Concentrations of PCBs in eggs were considered the most ecologically relevant endpoint to effects on reproduction induced by PCBs. Therefore, the assessment of potential risks based on TRVs derived from the exposure of eggs to PCBs was considered to be a maximum estimate of risk at all life stages [5]. Hazard quotients were calculated as the tissue concentration divided by the tissue-based TRV.

Toxicity reference values used to calculate hazard quotients were chosen for this study on the basis of several criteria, which included the use of wildlife species over traditional laboratory species whenever possible; chronic exposure including sensitive life stages; the evaluation of ecologically relevant endpoints; minimal cocontamination; multiyear studies; and total PCB or TEQ values were reported or could be calculated. Toxicity reference values were derived from several studies. Hudson River, USA, studies were included because they used the same species as this study and are the only studies known to observe reproductive effects attributable to exposure to PCBs [29] in tree swallows. Cocontaminants are also believed to be less than the threshold concentrations for effects. No laboratory studies have used tree swallows, and therefore this field study was deemed the most appropriate estimate of TRV values. Reproductive effects, including decreased hatching, increased abandonment, lower quality nests, and abnormal plumage coloration, were reported in tree swallows at PCB-contaminated sites relative to an uncontaminated area. In order to be protective of wildlife species, the Hudson River location with the lowest sitewide mean concentration of PCBs in eggs was chosen as the NOAEL. A NOAEL value of 26.7 μg PCB/g wet weight for eggs, nestlings, and adults was used. The $TEQ_{WHO-Avian}$ reported for eggs in that study was also used as the most conservative $TEQ_{WHO-Avian}$ estimate of the NOAEL (13 ng TEQ/g). The LOAEL for total PCBs was derived from a Housatonic River (Berkshire County, MA, USA) study in which hatching success was impaired during two years of the study [30]. The least concentration of total PCBs in pipping chicks from the two years (63 μg PCB/g) was used as the LOAEL. This study was deemed appropriate because effects were observed in two consecutive years and sensitive reproductive endpoints evaluated. A LOAEL based on $TEQs_{WHO-Avian}$ could not be derived from the literature.

RESULTS

Total PCB and organochlorine concentrations

Concentrations of PCBs in tree swallows were significantly different between sites. Concentrations of PCBs in adult tree swallows ranged from 0.44 to 32 μg PCB/g at TB and 1.0 and 2.0 μg PCB/g at FC. No statistically significant difference was observed in total PCB concentrations between eggs taken fresh or addled eggs at TB (two-way ANOVA, $p = 0.150$, $n = 13$) or FC (two-way ANOVA, $p = 0.423$, $n = 20$), although year was a significant factor at TB (two-way ANOVA, $p = 0.003$). Likewise, no statistically significant difference was observed in concentrations of PCBs between nestlings taken alive or salvaged carcasses at TB (Student's *t* test, $p = 0.290$, $n = 13$), so they were also combined for analysis. In both eggs and nestlings, a sixfold difference was observed between sites in mean concentrations of PCBs (Table 1). Mean concentrations of PCBs in nestlings and eggs from TB were both significantly greater than those at FC (Student's *t* test, $p < 0.001$). Concentrations of PCBs contained in nestling tree swallows ranged from 0.95 to 7.5 μg PCB/g at TB to a significantly smaller concentration at FC ranging from 0.14 to 2.0 μg PCB/g. Egg concentrations ranged from 1.2 to 15 μg PCB/g at TB and 0.18 to 2.5 μg PCB/g at FC.

Dichlorodiphenyltrichloroethane and its metabolites were detected in all samples from both sites. The isomer *p,p′*-dichlorodiphenyldichloroethylene (DDE) occurred at the greatest concentration of the measured analytes in all samples, which contributed 98% of the sum of total DDT concentrations in all samples. The sum of all measured isomers was used to calculate total DDT concentrations. Concentrations of total DDT at TB and FC were not significantly different (two-way ANOVA, location $p = 0.146$, condition $p = 0.384$, location × condition $p = 0.102$) because of large variances in the data, but the means were threefold different between sites. The mean (±1 standard deviation [SD]) concentration when all eggs were combined was 1.5 (1.2) μg DDT/g at TB and 0.45 (0.13) μg DDT/g at FC. Mean (±1 SD) concentrations in addled eggs

Table 1. Mean (±1 standard deviation) total polychlorinated biphenyl (PCB) concentrations (wet wt) and lipid content of tree swallow tissue samples from the Fort Custer State Recreation Area (reference location) and the former Trowbridge Impoundment within the Kalamazoo River area of concern, Michigan, USA

	Fort Custer			Trowbridge		
	n	% Lipid	PCB (μg/g)	*n*	% Lipid	PCB (μg/g)[a]
Egg						
2001	12	12 (7.1)	0.67 (0.40)	7	6.3 (2.3)	2.2 (0.74)
2002	7	8.5 (4.4)	1.0 (0.70)	7	8.2 (2.5)	8.1 (4.4)
Combined	19	11 (6.4)	0.81 (0.54)	14	7.2 (2.5)	5.1 (4.3)
Nestling						
2001	7	6.4 (2.4)	0.36 (0.16)	4	8.4 (2.6)	4.3 (2.3)
2002	5	8.0 (4.5)	0.59 (0.80)	7	8.1 (3.2)	2.6 (1.1)
2003	NA[b]	NA	NA	2	3.2 (0.060)	2.7 (0.59)
Combined	12	7.1 (3.3)	0.46 (0.51)	13	7.4 (3.2)	3.1 (1.6)
Adult						
2002	2	6.8 (0.33)	1.5 (0.65)	6	5.6 (2.5)	12 (11)
2003	NA	NA	NA	3	8.1 (3.8)	2.7 (3.1)
Combined	2	6.8 (0.33)	1.5 (0.65)	9	6.5 (3.0)	8.7 (9.7)

[a] All concentrations of PCBs in eggs and nestlings at the Trowbridge Impoundment were significantly greater than at the Fort Custer reference area (Student's *t* test, $p < 0.05$).
[b] NA = not available.

were 2.3 (0.32) μg DDT/g at TB and 0.32 (0.072) μg DDT/g at FC, and concentrations in fresh eggs were 0.95 (1.3) μg DDT/g at TB and 0.54 (0.030) μg DDT/g at FC.

$TEQ_{WHO-Avian}$ concentrations

Mean concentrations of $TEQs_{WHO-Avian}$ in eggs and nestlings at FC and TB were significantly different (Student's *t* test, $p < 0.001$) (Table 2). No statistically significant differences were observed between fresh and addled eggs at TB (two-way ANOVA, $p = 0.416$, $n = 11$) or FC (two-way ANOVA, $p = 0.137$, $n = 12$), although year was a significant cofactor at TB (two-way ANOVA, $p = 0.002$) also, no detectable differences were observed between live and salvaged nestlings at TB (Student's *t* test, $p = 0.378$, $n = 13$). Tree swallow eggs contained TEQ concentrations ranging from 0.21 to 2.4 ng TEQ/g at TB and 0.021 to 0.100 ng TEQ/g at FC. Nestlings at TB also had the greatest concentrations (0.26–1.3 ng TEQ/g) and nestlings at FC the lowest ($2.6 \times 10^{-3} - 0.047$ ng TEQ/g).

All adults from both locations had detectable concentrations of non-*ortho* PCBs. The average (±1 SD) $TEQ_{WHO-Avian}$ in adult tissues at TB was 2.2 ± 1.8 ng TEQ/g and 0.22 ± 0.030 ng TEQ/g at FC. An adult from TB had the greatest $TEQ_{WHO-Avian}$ for the study (4.8 ng TEQ/g).

Five of the eight mono-*ortho* congeners were regularly detected in samples from both TB and FC, but congeners 114, 123, and 189 were not detected in samples from either site. Samples were excluded from the analysis of $TEQ_{WHO-Avian}$ concentrations when at least one non-*ortho* congener was not

Table 2. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) and relative potency in tree swallow tissues sampled from the Fort Custer reference location and the former Trowbridge Impoundment within the Kalamazoo River Area of Concern, Michigan, USA

	Fort Custer			Trowbridge		
	n	TEQ (ng/g)	Relative potency (ng TEQ/g PCB[a])	*n*	TEQ (ng/g)[b]	Relative potency (ng TEQ/g PCB)
Egg						
2001	8	0.061 (0.030)	0.10 (0.035)	7	0.30 (0.11)	0.15 (0.069)
2002	4	0.045 (0.016)	0.078 (0.033)	5	1.4 (0.75)	0.16 (0.027)
Combined	12	0.056 (0.027)	0.095 (0.035)	12	0.76 (0.74)	0.15 (0.054)
Nestling						
2001	7	0.019 (0.016)	0.044 (0.027)	4	0.72 (0.40)	0.18 (0.094)
2002	5	0.023 (0.0085)	0.081 (0.058)	7	0.54 (0.16)	0.23 (0.066)
2003	NA[c]	NA	NA	2	0.57 (0.13)	0.21 (0.0029)
Combined	12	0.020 (0.013)	0.060 (0.044)	13	0.60 (0.25)	0.21 (0.069)
Adult						
2002	2	0.22 (0.030)	0.16 (0.048)	6	2.7 (1.7)	0.30 (0.26)
2003	NA	NA	NA	3	1.2 (1.9)	0.27 (0.23)
Combined	2	0.22 (0.030)	0.16 (0.048)	9	2.2 (1.8)	0.29 (0.23)

[a] Poloychlorinated biphenyls (PCB) defined as the concentration of the sum of 85 congeners with nondetects set at 0.5 of the detection limit.
[b] All concentrations of TEQs in eggs and nestings at the Trowbridge Impoundment were signficantly greater than at the Fort Custer reference area (Student's *t* test, $p < 0.05$).
[c] NA = not available.

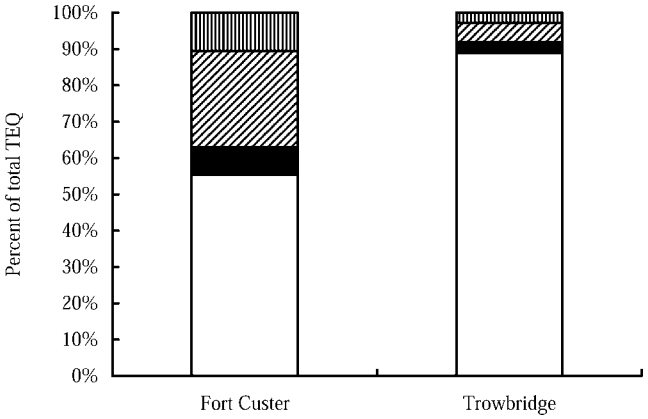

Fig. 2. Contribution of selected congeners to total dioxin equivalents (TEQ) in egg, nestling, and adult samples from the Fort Custer reference site and the Trowbridge Impoundment, Michigan, USA. ▥=Other; ▨=126; ■=81; □=77.

quantifiable because of interferences with coeluting congeners with the exception of PCB 169, which was not quantifiable in 65% of all samples. Congener 169 represented <1% of the total $TEQ_{WHO-Avian}$ concentrations in those samples for which it was reported. At least one of the coplanar congeners 77, 81, and 126 were not quantifiable because of coeluting congeners or were not above the detection limit in 71% of all samples at FC. Alternatively, only 11% of samples at TB had at least one of the three regularly detected coplanar congeners with concentrations that were not quantifiable or not detectable. For the remaining samples, the congeners detected at levels greater than the detection limit and the frequency of detection at TB were in the following rank order: PCB 77 = 105 = 118 (100%) > 157 (97%) > 81 = 156 (95%) > 126 (89%) > 167 (70%) > 169 (51%). Similarly, the rank order of the frequency of detection for mono-*ortho* and non-*ortho* congeners at FC was as follows: PCB 118 (100%) > 105 (97%) > 77 = 167 (86%) > 156 = 157 (77%) > 126 (66%) > 81 (43%) > 169 (20%). Coplanar PCB congeners 81 and 126 have the greatest AhR-mediated potency relative to other congeners and were detected in all TB nestling and adult samples and in 89% of egg samples at TB. Together they made up 8.3% ± 6.3 (mean ± 1 SD) of the total concentration of $TEQ_{WHO-Avian}$ in tissues at TB and 32.2% ± 25.6 of the total $TEQ_{WHO-Avian}$ at FC (Fig. 2). Congener 77 occurred at the greatest concentration in all matrices and contributed the greatest proportion to the total $TEQ_{WHO-Avian}$, 89.4% at TB and 67.8% at FC. Congener 167 was not reported in 22% of samples because of interference with coeluting congeners, but in the samples without interferences, congener 167 represented <1% of the total $TEQ_{WHO-Avian}$.

Biomagnification

Biomagnification factors calculated from lipid-normalized and wet-weight total PCBs and TEQs were less than 1.0 between egg and nestling and adult and egg but were greater than 1.0 between nestling and adult, except in 2001, when the egg-to-nestling BMF was greater than 1.0 at TB (Table 3).

Accumulation rates were calculated as the difference in total mass of PCBs and TEQs between nestlings and eggs in a nest divided by the days of life [31]. Both a nestling and an egg were sampled from five nests, allowing for the calculation of accumulation rates in those nests. Accumulation rates of 4.4 and 7.7 μg PCB/d were calculated for nests at TB during 2001 and 2002, respectively, and 0.90 to 1.1 μg PCB/d at FC for 2001 and 0.053 μg PCB/d at FC for 2002. Accumulation rates of $TEQ_{WHO-Avian}$ were 0.79 ng TEQ/d in 2001 and 1.2 ng TEQ/d in 2002 at TB, while accumulation rates of TEQs at FC in 2001 were 0.014 to 0.082 ng TEQ/d and 0.014 ng TEQ/d in 2002.

The relative contributions of non-*ortho* and mono-*ortho* congeners were evaluated by standardizing the TEQ to the total PCB concentration for each trophic level and comparing this relative potency between trophic levels via a potency ratio:

$$\frac{\left(\frac{\text{concentration of TEQs}}{\text{concentrations of total PCBs}}\right)_{\text{trophic level 2}}}{\left(\frac{\text{concentration of TEQs}}{\text{concentrations of total PCBs}}\right)_{\text{trophic level 1}}} \quad (1)$$

The potency ratio was calculated via methods outlined by Froese et al. [28] to describe changes in toxicity between trophic levels. Mean relative potencies (concentration of TEQs/concentration of total PCBs) used to calculate potency ratios are reported (Table 2). The egg-to-nestling potency ratio at TB and FC were 1.4 and 0.62, respectively. The ratios from nestling to adult and adult to egg were 1.3 and 0.52 at TB and 2.6 and 0.60 at FC, respectively.

Assessment of risk

Based on the NOAEL for both total PCBs and $TEQs_{WHO-Avian}$ (mean and 95% confidence limit), all HQs for egg, nestling, and adult tissue concentrations were all less than 0.3 at FC. Hazard quotients based on the mean and 95% upper confidence limit for eggs, nestlings, and adults at TB were also less than 1.0 for the NOAEL and LOAEL of total PCBs and the NOAEL for TEQs (Fig. 3). No samples from this study exceeded the LOAEL threshold, and only one adult sample from TB exceeded the NOAEL for total PCBs, yielding a HQ of 1.2.

Table 3. Biomagnification factors (BMFs) for tree swallows on the Kalamazoo River, Michigan, USA, based on the mean of lipid-normalized total polychlorinated biphenyls (PCBs) and dioxin equivalents (TEQs). Biomagnification factors based on wet-weight concentrations are reported in parentheses

	BMF (total PCB)				BMF (TEQ)			
	Fort Custer		Trowbridge		Fort Custer		Trowbridge	
	2001	2002	2001	2002	2001	2002	2001	2002
Egg to nesting	0.86 (0.50)	0.50 (0.89)	1.08 (1.98)	0.29 (0.47)	0.64 (0.90)	0.42 (0.51)	1.62 (2.71)	0.42 (0.39)
Nesting to adult	NA[a]	2.90 (2.80)	NA	7.01 (4.13)	NA	11.91 (10.52)	NA	8.07 (4.57)
Adult to egg	NA	0.68 (0.40)	NA	0.49 (0.51)	NA	0.20 (0.19)	NA	0.29 (0.56)

[a] NA = not available.

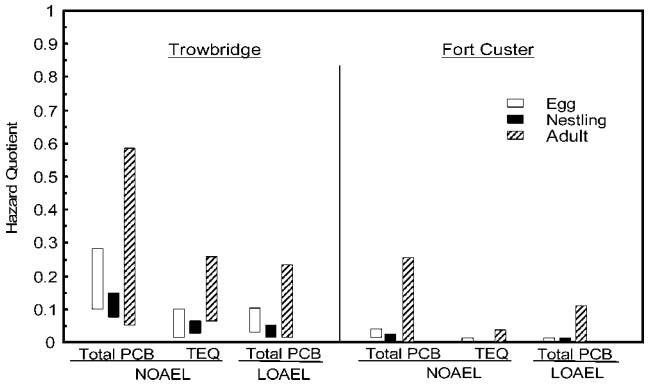

Fig. 3. Kalamazoo River, Michigan, USA, hazard quotients based on the no-observed-adverse-effect level (NOAEL) and the lowest-observed-adverse-effect level (LOAEL). Each box encompasses the 95% confidence interval about the mean. PCB = polychlorinated biphenyl.

DISCUSSION

Total PCB and DDT concentrations

Concentrations of PCBs in tree swallow eggs, nestlings, and adults at the FC reference site were significantly less than those at the TB site. Reference sites on the Thompson River, Fraser River, Fox River, and around the Great Lakes, USA, had PCB contaminant levels in tree swallow tissues within a twofold range of those found at FC with no corresponding reproductive effects [19,32–34].

With one exception, concentrations of PCBs found in tree swallows from the KRAOC were generally similar to or less than at other sites in the Midwest and New York, USA, where PCBs have been identified as a chemical class of concern [18,19,33]. Mean concentrations of PCBs in eggs and nestlings from the KRAOC were 5.1 μg PCB/g and 3.1 μg PCB/g, respectively. Tissues from tree swallows at the Fox River near Green Bay, Wisconsin, USA, were most similar to those at the KRAOC with mean concentrations of PCBs in eggs and nestlings as great as 4.1 and 3.0 μg PCB/g, respectively [32]. Although approximately 200 km from the KRAOC, tree swallows from Saginaw Bay, Michigan, USA, contained lesser tissue concentrations of 0.60 to 1.4 μg PCB/g in eggs and 0.17 to 1.0 μg PCB/g in nestlings [35]. One egg concentration reported from the Great Lakes was 10-fold greater than the greatest egg concentration at the KRAOC, and a nestling concentration was eightfold greater than the greatest concentration at our site [19]. To date, the greatest concentration of PCBs reported in eggs or pipping chicks was at the Housatonic River, Massachusetts, USA (101 μg PCB/g) [30], and the greatest concentration in nestlings was reported at the Hudson River (62.2 μg PCB/g) [33]. Concentrations of PCBs at both sites were 20-fold greater than at the KRAOC. In addition to concentrations of PCBs in eggs and nestlings, few studies besides the current study have reported tissue concentrations in adults based on more than a few incidentally salvaged samples. In a single adult female, a PCB concentration of 114 μg PCB/g was reported at one Hudson River site [33]. That concentration was considerably more than for adults at TB (mean = 8.7 μg PCB/g).

Sources of DDT and its isomers at the KRAOC are unknown but are likely related to former agricultural practices and the prevalence of orchards in downstream portions of the Kalamazoo River drainage system. Although DDT concentrations were greater at the TB site than at the FC reference site, they were not sufficient to affect the viability or hatching success of tree swallow eggs. Hatching and overall productivity were comparable between the two sites, and no unusual instances of shell breakage were noted at either of the Kalamazoo River populations studied [7]. A concentration of 2.0 μg DDE/g in eggs has been suggested as a threshold for effects in raptor species, which was similar to the mean for the TB population [36]. Concentrations of DDE in the abandoned eggs of tree swallows near Denver, Colorado, USA, were 2.8 μg DDE/g, while concentrations of DDE in attended eggs were 1.3 μg DDE/g [37]. This study was designed to compare a reference location with relatively low concentrations of PCBs in the sediment to a location with greater concentrations of PCBs. When differences in reproduction are present, it is not possible to make a determination of causality for those differences, whether it is DDE, PCBs, or another factor. When no differences in reproductive success are present between locations, as is the case for the Kalamazoo River, no differential factors existing at the locations are comparatively impairing reproduction. Concentrations of DDE may be near a threshold for effects at both locations and may influence reproduction at both locations compared to populations of tree swallows at other locations, but DDE concentrations do not appear to influence success at significantly different levels between the two populations.

TEQ tissue concentrations

If concentrations of TEQs and those of total PCBs were correlated, it would be expected that few AhR-active compounds other than PCBs were contributing to the total TEQ concentration [18]. Although they were calculated with different TF, the calculated $TEQs_{WHO-Avian}$ for tree swallows at the KRAOC were similar to those TEQs calculated for other PCB contaminated sites in the midwestern United States [17,18,28]. Concentrations of $TEQs_{WHO-Avian}$ in eggs, nestlings, and adults from the KRAOC were 10 to 30-fold greater than concentrations at the reference site, compared to a three- and 10-fold difference in concentrations of $TEQs_{WHO-Avian}$ between reference sites and contaminated sites at the Thompson and Fraser Rivers, respectively [34]. Concentrations of $TEQs_{WHO-Avian}$ in nestling tree swallows at the FC reference site were at least fourfold greater than the upstream reference sites on the Thompson and Fraser Rivers, USA, and were most similar to the downstream site of the Thompson River [34].

Besides the statistically significant difference in total $TEQs_{WHO-Avian}$ between sites, a difference was also observed in the proportion that each congener contributed to the overall $TEQ_{WHO-Avian}$. As has been observed at other sites [33,38], congeners 77 and 126 contributed the greatest relative proportion of the total $TEQ_{WHO-Avian}$ concentrations at both FC and TB. However, the contribution of PCB congener 77 was significantly greater at TB in all life stages than at FC (Student's t test, $p < 0.001$). In contrast, PCB congeners 81 and 126 contributed a significantly greater proportion to the total $TEQs_{WHO-Avian}$ at FC when evaluated together or as individual components than at TB (Student's t test or Kruskal–Wallis, $p < 0.05$). The prevalence of PCB congener 77 at TB was likely due to the presence of this congener in the original Aroclor mixture, while the proportion of PCB congeners 81 and 126 comprising the $TEQs_{WHO-Avian}$ at FC could be attributed to the greater $TEQs_{WHO-Avian}$ of these congeners. Other mono-*ortho* congeners also contributed a greater proportion of the total $TEQ_{WHO-Avian}$ at FC than at TB, but the overwhelming presence

of PCB congener 77 at TB likely contributes to this observation.

Biomagnification

Lipid-normalized and wet-weight biomagnification factors followed similar trends. The BMF from egg to nestling was less than from nestling to adult. This is due to growth dilution of the chicks and fugacity effects in the adults [18]. The egg-to-nestling BMFs would be expected to exceed 1.0 if nestlings are exposed to concentrations of PCBs in the diet that are greater than would be necessary to offset growth dilution. The BMF above 1.0 observed in tree swallow nestlings from TB in 2001 suggests a dietary exposure. The contribution of sitewide contamination during the nestling stage can be assessed because the sedentary behavior of altricial tree swallow nestlings and the relatively small feeding range of the adults ensures that dietary exposure—and subsequent accumulation in chicks—results from on-site dietary exposure. Therefore, the egg-to-nestling BMF is very informative when evaluating the bioaccumaltive potential of a toxicant at a specific site. Overall, the Kalamazoo River egg-to-nestling BMFs were less than those found at the Fox River (6.18 and 3.47) [32] but were comparable to those on the Hudson River (2.0 and 4.0) [33].

The greatest increase in bioaccumulation was between nestlings and adults for lipid-normalized and wet-weight values. Fort Custer accumulation factors were generally greater than at TB, likely resulting from the low concentrations of PCBs at the site, especially in nestlings. Concentrations near the detection limit may be difficult to quantify because of baseline noise, and so the difference in concentrations of PCBs in the nestling and adult tissue is great. When evaluating the nestling to adult BMF, the contribution of off-site exposure and also maternal deposition in the lipids of eggs needs to be considered. At the KRAOC, most female birds were captured post-reproduction. Perhaps dietary contributions to the body burdens of adults had already accumulated to prereproductive levels when sampling was conducted, or the loss of body burden to maternal deposition was not significant in our population since most females were captured postreproduction. Therefore, concentrations of PCBs in adults were greater than in nestlings because of on- and off-site exposure as well as the lack of growth dilution.

Bioaccumulation of PCBs was also evaluated on the basis of daily accumulation rates, which accounted for the age of nestlings and the concentration contributed by the egg [31]. The calculation of daily accumulation rates is useful in elucidating whether nestlings were exposed to concentrations of PCBs from the diet. Accumulation rates at TB (4.4 and 7.7 μg PCB/d) were similar to those reported for contaminated sites at the Fox River (1.34–6.69 μg PCB/d) [32] but were less than those measured at the Housatonic River (34–76 μg/d) [30]. Accumulation rates at FC (0.053–1.1 μg/d) were similar to the reference site at the Housatonic River (−0.30–1.0 μg/d) [30] and contaminated sites at the Wisconsin River (0.40–0.70 μg PCB/d) [38]. The accumulation rate in KRAOC tree swallows is less than in piscivorous birds, such as the Forster's tern (*Sterna forsteri*), which had accumulation rates of 15 μg PCB/d [17].

Potency ratios were generally greater than 1.0 for both the egg to nestling and nestling to adult, which suggests that the contribution of non-*ortho* and mono-*ortho* congeners to the overall toxicity increased at higher trophic levels. A ratio of 1.0 between adult and egg was expected because little metabolism or growth dilution occurs in the egg, and the egg burdens are inherited from the adult. However, at the KRAOC, potency ratios were 0.60 and 0.52. This suggests that congeners contributing the greatest concentrations to the relative toxicity did not pass into the egg. Bioaccumulating congeners in the penta-, hexa-, and hepta-homologue groups contribute the greatest amount to the total PCB concentration in adult tree swallows, whereas less chlorinated congeners in the tri- and tetra-homologue groups contribute a lesser amount (Fig. 4). The accumulation of these congeners likely led to the greater relative potency and overall toxicity of the congener mixture found in adults. A greater proportion of total PCBs in eggs and nestlings was contributed by the tri- and tetra-homologue groups and a lesser proportion by the more chlorinated homologue groups. The greater concentration of bioaccumulating congeners in the adults and the greater relative potency in the adults suggests that the toxicity of PCBs in the KRAOC increases with trophic level, and the adults contribute a metabolized, relatively less toxic mixture to the eggs. Overall, adults appear to contain greater proportions of bioaccumulating congeners, and the mixture of congeners in adults is more toxic than in earlier life stages. The proportionate contribution of congeners in eggs and nestlings is similar, but the toxicity of the mixture increases with life stage.

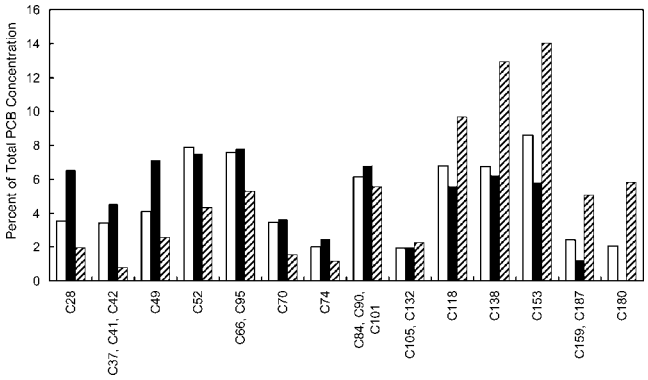

Fig. 4. The percent contribution of selected congeners and congener coelution groups to the concentration of total polychlorianted biphenyls (PCBs) in egg, nestling, and adult tree swallows at the Trowbridge Impoundment, Michigan, USA. □=Egg; ■=nestling; ▨=adult.

Assessment of risk

Hazard quotients of less than 1.0 suggest that tissue concentrations in all stages of the tree swallow life cycle were less than the threshold for effects based on the LOAEL. In most instances, even based on the more conservative NOAEL, HQs were less than 1.0 for all tissues (Fig. 3). It should be noted that the true effect level for individuals lies somewhere between the NOAEL and LOAEL and, even conservatively, population effects are not expected at a HQ of 10.0. Thus, while a few individuals did have NOAEL HQ values between 1.0 and 2.5, reproductive dysfunctions would not be expected, nor were any seen [7]. Tree swallows on the Hudson River had concentrations of PCBs that were 10-fold greater than the concentrations observed at the KRAOC, and reproductive effects were barely identifiable above the variation naturally inherent in field studies [33].

Multiple lines of evidence, including both exposure and effects, were explored to quantify risk to passerine species with an aquatic-based diet. Both the total PCB and the TEQ

method of evaluating exposure concentrations resulted in similar conclusions of little to no risk to tree swallow species at the KRAOC. Even when the most conservative TRV criteria were used, tissue concentrations at the site suggest that only those individuals with the greatest exposure may be at risk for effects attributable to PCBs, but for the population as a whole, the current concentrations of PCBs are less than the threshold for effects. Accordingly, other studies with similar tissue concentrations did not observe reproductive effects attributable to on-site exposure to PCBs. In short, multiple lines of evidence based on three years of observation find that KRAOC tree swallow populations are not affected by PCB contamination at an ecologically relevant level [7].

CONCLUSION

Prior to this study, little information was available on the extent of PCB exposure and potentially related effects on Kalamazoo River passerine birds. Passerines are not only important ecological receptors in their own right but also a potential prey component of higher food web receptors within the Kalamazoo River basin, including the great horned owl (*Bubo virginianus*) and bald eagle (*Haliaeetus leucocephalus*). Elevated concentrations of PCBs in TB passerine nestlings indicate environmentally available concentrations of PCBs in the vicinity of the TB nests. The PCB concentrations contained in adults and eggs indicate the presence of life cycle body burdens, which are critical for evaluation of the health and sustainability of passerine populations. Here, we used site-specific data to perform a risk-based evaluation of passerine health and sustainability in PCB-contaminated areas of the Kalamazoo River. The evaluation directly compared exposure and productivity data for the target area to that of the FC reference area of the Kalamazoo River. Concentrations of PCBs were significantly greater in tree swallows from the KRAOC than those from the upstream reference site, and they were comparable to other PCB-contaminated sites in the region. Based on a three-year, site-specific, multiple-lines-of-evidence approach, tree swallows from the KRAOC region of the Kalamazoo River were exposed to PCBs at concentrations that were significantly greater than similar areas without point-source inputs. However, these exposures were less than those expected to cause population-level effects, and no effects were observed in Kalamazoo River tree swallow populations.

Acknowledgement—The authors would like to extend a special thanks to the students and technicians at the Michigan State University Aquatic Toxicology Laboratory and the Kalamazoo Nature Center, including M. MacCarroll, C. Joldersma, B. Joldersma, R. Holem, M. Kramer, K. Smyth, and K. Coady. Funding was generously provided by the Kalamazoo River Study Group.

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